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# Evaluation of the protective effect of human cytotoxic lymphocytes (CTL) specific for P210$^{BCR-ABL}$ protein in a SCID mouse model

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Chronic myelogenous leukemia (CML) cells are characterized by a specific (9,22) translocation which encodes for the chimeric bcr-abl fusion 210kD oncoprotein (P210$^{BCR-ABL}$). Some peptides from the breakpoint fusion are able to bind to major histocompatibility complex class I molecules and thus to generate CTL which are specific for BCR-ABL peptide. CTL specific for the HLA-A201 restricted CTL epitope SSKALQRPV (926-934) were generated from peripherical blood mononuclear cells from a healthy HLA-A201 donor. We studied the *in vivo* protective effect of passive transfers of CTL lines specific P210$^{BCR-ABL}$ protein on tumor progression in a SCID mouse model. Subcutaneous inoculation of K562 cells transfected with HLA A201 gene was able to induce hypodermic BCR-ABL tumor into SCID mice. NK-depleted specific CTL lines, injected directly into the tumoral site, delayed the appearance of the tumor and decreased K562-A201 tumor weight for at least 50%, while no effect was observed on the control K562 tumor. It is the first *in vivo* demonstration of the antitumoral effect of human CTL specific for BCR-ABL in a SCID mouse model. Immunotherapy with specific CTL or peptide-based vaccination could be a new interesting therapeutic approach for CML therapy.

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# Dendritic cell-derived exosomes elicit potent anti-tumor immune responses *in vivo*

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**Abstract.** Dendritic cells (DCs) are professional antigen presenting cells, involved in the priming of T cell-mediated immune responses. We now show that in addition to direct cell-cell contacts and to the production of various cytokines, DCs may also trigger T cell responses through the secretion of antigen presenting vesicles, called exosomes. Exosomes represent the internal vesicles of multivesicular endosomes, which are secreted after fusion of the external membrane of endosomes with the plasma membrane. Human and mouse DCs secrete exosomes bearing endosomal markers absent from the cell surface, such as CD63 and CD82. DC-derived exosomes also express MHC class II and B7.2 co-stimulatory molecules, as well as functional MHC class I molecules, which stimulate antigen-specific CD8+ T cell clones *in vitro*. Importantly, exosomes

produced by tumor peptide-loaded DCs prime specific cytotoxic T lymphocytes *in vivo* and eradicate established tumors. In addition, the secretion of exosomes by DCs is upregulated by IFNg, IL-12 and IL-10, but not by TNFα or LPS, which efficiently induce DC maturation. The potent antitumor immune response induced by exosomes supports their implementation for cancer immunotherapy. In addition, exosomes may represent a novel "liposome like" means of communication between cells of the immune system.

**Effet antitumoral des exosomes produits par les cellules dendritiques**

**Résumé.** Les cellules dendritiques sont des cellules présentatrices d'antigènes impliquées dans l'initiation des réponses immunitaires. Nous avons montré que les cellules dendritiques sécrètent des vésicules membranaires appelées exosomes. Les exosomes correspondent aux vésicules internes des endosomes multivésiculaires (ou endosomes tardifs), qui sont sécrétés dans le milieu extra-cellulaire lorsque les endosomes tardifs fusionnent avec la membrane plasmique. Les exosomes expriment des molécules de classe I et II du Complexe Majeur d'Histocompatibilité (CMH) et sont capables de stimuler directement des lymphocytes T de manière antigène spécifique. Des exosomes produits par des cellules dendritiques préalablement sensibilisées avec des peptides issus de tumeurs, induisent la régression ou la disparition de tumeurs établies dans différents systèmes expérimentaux. De plus, la production d'exosomes est régulée positivement ou négativement par différentes cytokines. Les exosomes représentent donc un nouveau vecteur acellulaire de vaccination anti-tumorale. Ils pourraient, par ailleurs, être considérés comme des liposomes naturels, impliqués dans la communication entre cellules du système immunitaire.

Key words: Exosomes – Dendritic cells – Tumor vaccination

Dendritic cells (DCs) are professional antigen presenting cells, involved in the priming of T cell-mediated immune responses [revue in 1]. DCs have the unique capacity to initiate both primary and secondary immune responses in vivo. DCs exist under two distinct functional states: i) the immature state is characterized by high endocytic capacity, abundant MHC molecules in cytoplasmic vesicles of endocytic origin and low T cell co-stimulatory activity. Over all immature DCs are very efficient for ingestion and processing of antigens, but very inefficient for T cell activation. ii) a mature state, where the endocytic activity is down regulated, whereas expression of MHC molecules and T cell co-stimulatory molecules are increased. Mature DCs are therefore not capable of antigen ingestion and processing, but may efficiently present peptides derived from antigens internalized under their immature state, to specific T lymphocytes. Transition between the immature and mature states is induced by inflamation cytokines, such as TNFα or IL1, as well as by compounds of the bacterial cell wall, such as lipopolysaccharide (LPS). Induction of maturation also triggers DCs migration from peripheral tissues towards lymphoid secondary organs, such as lymph nodes, where activation of naive or memory T cells occurs. This activation occurs through direct cell-cell contact and the secretion of specific cytokines.

We now show that in addition to direct cell-cell contacts and cytokine production, DCs may also trigger T cell responses through the secretion of antigen presenting vesicles, called exosomes. Exosomes represent the internal vesicles of mutivesicular endosomes, which are secreted after fusion of the external membrane of endosomes with the plasma membrane. Raposo et al. [3] have previously shown that human Epstein-Barr virus (EBV) transformed B lymphocytes secrete exosomes, which bear functional HLA-DR molecules. In the case of B lymphocytes, exosomes represent the internal vesicles of a subpopulation of MHC class II compartments. Similar compartments have been described in DCs [2].

Human and mouse DCs secrete exosomes in the culture supernatants. Exosomes from DCs can be purified from cellular supernatants by differential ultracentrifugation. Purified exosome preparations contain homogenous populations of membrane vesicles of 60-90 nm diameter. The capacity to produce exosomes is down-regulated upon DC maturation (unpublished results). Thus, DCs treated with LPS or TNFα did not secrete exosomes any longer. In contrast, treatement with IL-10, IFN-γ or IL-12 increased by 4-6 folds the amount of exosomes produced by DCs. Other cytokines like IL-4, IL-2 or IL-5 had no effect on exosome production.

DC-derived exosomes bear endosomal markers absent from the cell surface, such as CD63 and CD82. They also express MHC class I and MHC class II molecules as well as and B7.2 co-stimulatory molecules. Exosomes produced by DCs incubated with antigenic peptides stimulate the corresponding antigen-specific CD8+ T cell clones *in vitro* (L. Zitvogel et al., submitted).

Importantly, exosomes produced by tumor peptide-loaded DCs primed specific cytotoxic T lymphocytes in

vivo and eradicate established tumors. In collaboration with the group of L. Zitvogel (IGR, Paris), we tested the anti-tumor effects of exosomes in two tumor models. P815 is an immunogenic but very agressive mastocytoma, syngeneic of DBA/2 (H-$2^d$), for which very few effective immunotherapies on day 10 established tumors have been reported. TS/A is a poorly immunogenic, spontaneous mammary carcinoma, expressing lower levels of MHC class I molecules, syngeneic of BALB/c (H-$2^d$).

Acid eluted tumor (P815 or TS/A) peptides were pulsed onto syngeneic mouse bone marrow-derived (BM-DCs) as previously described [4]. Exosomes were prepared from the DC supernatants and utilized for in vivo immunization. Therapy of day 10-established P815 tumors (50-90 $mm^2$ in size) was carried out using a single intradermal (id) administration of 3-5 mg of exosomes/mouse. Within a week, tumor growth stopped in the groups treated with exosomes derived from autologous tumor peptide pulsed DCs, and 40 to 60% mice were tumor free at day 60. These animals had a long lasting immune response and rejected a lethal tumor challenge with P815, but not with the syngeneic leukemia L1210. Therefore, P815 peptide-pulsed DC derived-exosomes promoted complete tumor regression or significant retardation in most treated animals (L. Zitvogel et al., submitted).

Similar antitumor effects were achieved with day 3-4 established TS/A tumors. In this setting, all mice had statistically significant tumor growth delay that prolonged their survival. Preliminary results show that lung metastases, were not detectable by two different pathologists, in d40 exosome treated mice. These antitumor effects were not found in athymic Nu/Nu counterparts injected in parallel with the same exosome preparations, indicating that T cells are required for the exosome-induced antitumor immune responses.

In addition, exosomes directly primed tumor specific CTL responses in P815 bearing hosts. Splenocytes from mice that rejected P815 tumors following immunization with exosomes were harvested at day 90 and cultured for 5 days in the presence of irradiated B7.1-expressing P815 cells to enhance specific precursor frequency. Therefore, a single injection of exosomes derived from DCs pulsed with the relevant peptides efficiently primed specific antitumor CTL responses *in vivo*.

All together, our results strongly support the implementation of DC-derived exosomes for cancer immunotherapy. As cancer vaccines, exosomes associate the advantages of DCs (association of MHC class I and II together with T cell costimulation) and of cell-free vectors. Clinical use of exosomes will, however, require their extensive biochemical characterization and the analysis of the mechanisms underlying their bioactivities.

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